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Adhesion of Type A *Pasteurella multocida* to Rabbit Pharyngeal Cells and Its Possible Role in Rabbit Respiratory Tract Infections

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Pasteurella multocida serotype A was found in association with the mucosal epithelium of the nasopharynges of rabbits with respiratory tract infections. The bacteria specifically attached to squamous epithelial cells of the pharyngeal mucosa both in vivo and in vitro and to some tissue culture cell lines such as HeLa. All strains with serotype A capsules were adhesive. With the exception of one serotype D strain, strains with capsular serotypes B, D, and E were at least 10-fold less adhesive. Bacterial adhesiveness was much reduced after pronase digestion, heat treatment, and homogenization, but removal of the hyaluronic acid capsule increased adhesion. Electron microscopy revealed that fimbriae were produced by an adhesive pasteurella strain, but not by two nonadherent strains. The attachment of the former strain to pharyngeal and HeLa cells was inhibited by *N*-acetyl-D-glucosamine. Together, these findings suggest that this amino sugar may be a component of the receptor on both animal cell surfaces and that the fimbriae may be the adhesins. It is proposed that bacterial attachment has a role in colonization and infection of rabbit upper respiratory mucosae.

Pasteurella multocida is a member of the normal flora of mucosal surfaces (17, 18) and a widely distributed pathogen of many animal species (2, 4). Of the four capsular serotypes of *P. multocida*, serotypes A and D cause primary and secondary infections of several species and are those most frequently implicated in chronic upper respiratory tract disease and pneumonia in rabbits (2, 5, 6). Serotypes B and E, the agents of hemorrhagic septicemia in cattle, are not found in North America (2).

Pasteurellae colonize the mucosal surfaces of the pharynges of rabbits in the carrier state or with recurrent rhinitis (5, 11, 16). In the early stages of experimentally induced disease produced by intranasal inoculation of pathogenic strains of *P. multocida* (16), pasteurellae can be isolated only from the tissue surfaces of the naso- and oropharynx. Subsequently, ascending infections of the sinuses and middle ears are observed (14). These findings suggest that *P. multocida* proliferates initially on the nasopharyngeal mucosae and that this may be an important aspect of the pathogenesis of rabbit pasteurellosis. Some bacteria are known to colonize mucosal surfaces because they produce adhesive substances (1), and colonization of the nasopharyngeal mucosae by pasteurellae may also involve adhesive mechanisms. We have studied, therefore, the adhesion of pasteurellae to rabbit pharyngeal and tissue culture cells and present evidence on the possible nature of the bacterial adhesin and its receptor.

MATERIALS AND METHODS

Bacterial strains and culture. *P. multocida* strain R-1 (serotype 3,12:A) was used as a reference strain in all experiments. This strain was isolated originally from the respiratory tract of a rabbit with rhinitis (8). Other strains, representing the four capsular serotypes, are described in the results (see Table 2). These strains were isolated from several animal species with clinical pasteurellosis. Some of these isolates were serotyped and provided by G. Carter, Michigan State University, East Lansing; R. Rimler and K. Rhoades, National Animal Disease Center, Ames, Iowa; Yue-Shoung Lu, Southwest Medical School, Dallas, Tex.; and L. Spanoghe, Laboratory for Microbiology and Infectious Diseases, Rijksuniversiteit, Gent, Belgium.

Bacteria were grown routinely as static cultures in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) at 37°C for 18 h, collected by centrifugation (8,000 × *g*, 10 min), suspended in Hanks balanced salt solution (HBSS; GIBCO Laboratories, Grand Island, N.Y.) containing 0.01 M HEPES buffer (H), pH 7.4, and 0.035% sodium bicarbonate (HBSS-H), and sedimented again by centrifugation. Bacteria were resuspended in HBSS-H containing 10% fetal calf

serum (FCS), and total bacterial numbers were adjusted to the desired concentration by using a Petroff-Hausser chamber (Hausser Scientific, Blue Bell, Pa.).

Experimental rabbit infections and the association of *P. multocida* with the respiratory tract mucosae. Strain R-1 was inoculated intranasally (10^{10} bacteria in 1 ml of saline) into pasteurella-free New Zealand white rabbits (Dutchland Laboratories, Denver, Pa.) on a daily basis until clinical signs of disease appeared. Diseased rabbits were killed by CO_2 asphyxiation.

Cryostat sections were prepared from portions of the oral and nasal pharynges and fixed in methanol. The mucosae of the remaining portions of the pharynges were scraped with a sterile scalpel to remove pharyngeal cells. The cells were suspended in HBSS and washed four times by repeated centrifugation (50 × *g* for 5 min). Samples of the cell suspension were spread on microscope slides and fixed with methanol. Tissue sections and smears were stained with May-Grünwald-Giemsa stain and examined for the presence of adherent bacteria. Adherent bacteria on other preparations were identified as *P. multocida* by indirect immunofluorescence with specific anti-pasteurella serum.

The numbers of pasteurellae in homogenates of mucosal cell suspensions were quantitiated by using standard plate counts. *P. multocida* was identified by using standard techniques (Analytab Products, Inc., Plainview, N.Y.).

Preparation of pasteurella-specific antiserum. Germ-free rats (Charles River Breeding Laboratories, Wilmington, Mass.) were injected intradermally with lyophilized, Formalin-fixed whole *P. multocida* strain R-1 at a dose of 1 mg/kg of body weight in polyadenylic-polyuridylic acid adjuvant (0.25 mg/kg, Sigma Chemical Co., St. Louis, Mo.). Antibody that cross-reacted with bacteria of the normal flora of the rabbit respiratory tract was removed by repeated adsorption (H. G. Rush, J. C. Glorioso, C. A. DaRiff, and L. C. Olson, Am. J. Vet. Res., in press). Bacterial species commonly isolated from the rabbit respiratory tract included *Escherichia coli, Pseudomonas fluorescens* and *P. aeruginosa, Neisseria catarrhalis, Micrococcus luteus, Staphylococcus epidermidis, Bacillus cereus* and *B. subtilis, Bordetella bronchiseptica*, and *Acinetobacter anitratus*. The absorbed antiserum reacted with strain R-1 in the indirect immunofluorescence test, but did not react with bacteria of the normal rabbit respiratory tract in situ or react after isolation.

In vitro adhesion of *P. multocida* to rabbit pharyngeal cells. Naso- and oropharyngeal epithelial cells were obtained from healthy rabbits killed by CO_2 asphyxiation and suspended in erythrocyte lysis buffer (0.155 M NH_4Cl–0.01 M $KHCO_3$–0.1 mM EDTA, pH 7.4) for 10 min at 4°C to remove erythrocytes. The cells were washed three times in HBSS plus 10% FCS by centrifugation. Mature squamous and ciliated epithelial cells had readily identifiable morphologies.

Mixtures of 10^6 epithelial cells and 10^9 bacteria in 1 ml of HBSS-FCS were incubated at 37°C for 2 h with constant agitation by using a rocker platform (Bellco Glass, Inc., Vineland, N.J.) set at 10 oscillations per min. The epithelial cells were centrifuge-washed four times in HBSS-FCS to remove nonadherent organisms, spread on glass slides, fixed with methanol, and stained with May-Grünwald-Giemsa stain. The mean and standard deviation (SD) of the numbers of adherent bacteria on each of 25 randomly selected epithelial cells were determined, and pairwise comparisons of individual means were made with the two-sample Student *t* test. Controls included cells incubated without pasteurellae.

In vitro adhesion of *P. multocida* to tissue culture cells. HeLa, primary rabbit kidney (15), primary human embryonic lung, African green monkey kidney (Vero) and baby hamster kidney (BHK-21) cells were grown in Eagle minimum essential medium containing 10% FCS and 100 U of penicillin and streptomycin per ml (GIBCO).

Nonconfluent monolayers (approximately 3×10^5 cells per monolayer) on glass cover slips were washed with HBSS-H and then incubated at 37°C with 1-ml suspensions of bacteria (10^9/ml) for 30 min. Nonadherent bacteria were removed from the monolayer with two 3-ml rinses of HBSS-H, and the monolayers were stained with May-Grünwald-Giemsa stain. The mean and standard deviation of the number of bacteria adhering to each of 25 randomly selected cells were determined and analyzed as above. For tabulation purposes, results from all replicate experiments were averaged after normalization to their respective controls according to the formula: mean number of adherent bacteria per animal cell in test condition/mean number of adherent bacteria per animal cell in control condition × 100.

The effect of bacterial growth conditions and enzymatic and physicochemical treatments of pasteurellae on adhesion. Early exponential-, midexponential-, and stationary-phase bacteria (3, 8, and 24 h, respectively) grown in static cultures—cultures grown with agitation or grown anaerobically in Brewer jars (The Torsion Balance Co., Clifton, N.J.)—were tested for their ability to attach to HeLa and pharyngeal cells. The influence of culture media on adhesive properties was examined with bacteria grown in brain heart infusion broth containing 0.5% dextrose, 5% Bacto-Fildes enrichment, or 50% normal rabbit serum (pasteurella antibody-free) or on brain heart infusion agar, Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) with 10% sheep blood (blood agar), dextrose starch agar, and chocolate blood agar (Rupp and Bowman Co., Detroit, Mich.). All cultures were grown at 37°C overnight. Bacteria were washed from the agar surface with HBSS-H or collected from the broth by centrifugation and suspended in HBSS-H.

The hyaluronic acid capsules (2, 9) were removed from *P. multocida* by incubating a sample of the bacterium in Dulbecco phosphate-buffered saline (pH 6.0) containing 10 NF units of hyaluronidase (Wyeth Laboratories, Philadelphia, Pa.) for 2 h (3). Decapsulation was confirmed by negative staining. Bacteria were also treated with either pronase (Sigma) at a concentration of 100 μg/ml in HBSS, pH 7.4, for 1 h at 37°C, lipase (Sigma) at 100 μg/ml in HBSS, pH 7.4, or mixed glycosidases (Miles Laboratories, Elkhart, Ind.) at 200 μg/ml in HBSS, pH 7.4, for 1 h at 37°C or with 0.05% Nonidet P-40 (Sigma) for 1 h at room temperature. Bacteria were subjected to hydrodynamic shear by homogenization in a Waring blender for 15 min or to heat treatment at 100°C for 10 min.

Adhesion inhibition test, using various carbohydrates. Adhesion inhibition assays were carried out in the presence of L-fucose, D-galactose, *N*-acetyl-D-

glucosamine, *N*-acetylneuraminic acid, D-mannose, or D-glucose (Sigma) at concentrations of up to 5 mg/ml in HBSS, pH 7.4. Suspensions of strain R-1 were incubated with each of these sugars for 15 min at 37°C, and the mixtures of bacteria and test sugars were added to HeLa cell monolayers or to rabbit pharyngeal cell preparations.

Electron microscopy. Stationary-phase *P. multocida* strains R-1, 656, and Bunia II were washed in normal saline and negatively stained with 2% sodium phosphotungstate, pH 6.8. Preparations were examined by using a Phillips EM201 electron microscope.

RESULTS

The association of *P. multocida* with the pharyngeal mucosa of diseased rabbits. Few bacteria were recovered from mucosal scrapings of the pharynges of healthy control rabbits (10^2 to 10^3 bacteria per 10^6 total mucosal cells), and pasteurellae were never isolated. Animals experimentally infected with strain R-1 showed clinical signs of rhinitis 2 days after infection, and some animals developed severe clinical signs of respiratory disease (as evidenced by purulent nasal discharge and labored breathing), which sometimes culminated in pneumonia by 1 week. Infected rabbits harbored large numbers of *P. multocida* in their pharynges (10^6 to 10^7 pasteurellae per 10^6 total mucosal cells).

Fluorescent-antibody staining of cryostat sections of the pharyngeal mucosa revealed *P. multocida* closely associated with the tissue surface (Fig. 1).

Preparations of mucosal cells were composed of 30% mature parakeratotic squamous epithelial cells, 65% less-differentiated columnar cells and cells from other strata in the stratified epithelium, and 5% ciliated cells. Pasteurellae were found attached (mean, 14 ± 17 bacteria per cell) to mature isolated pharyngeal squamous cells (Fig. 2), but not to ciliated epithelial cells or to other less-differentiated cell types. Approximately 5% of squamous cells had bacteria attached in numbers too numerous to quantitate (>200 bacteria per cell).

Adhesion of *P. multocida* to normal rabbit pharyngeal cells and to tissue culture cells in vitro. Broth-grown *P. multocida* also adhered to squamous pharyngeal cells in vitro, but not to cilia of ciliated cells (Table 1). Maximal numbers attached when a 1,000:1 ratio of bacteria to

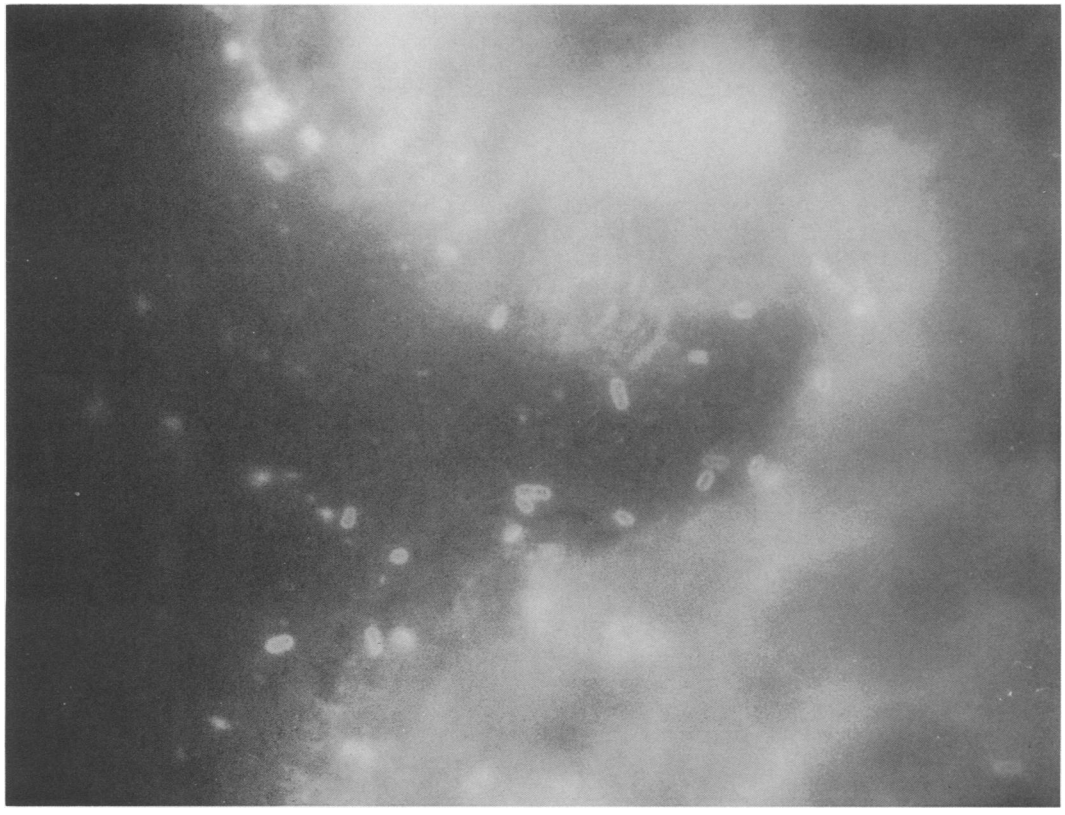

FIG. 1. Tissue section of a rabbit pharynx with *P. multocida* adhering to the mucosal surface. The pharynx was removed from a rabbit with a respiratory tract infection, frozen at −70°C, sectioned, fixed, and stained by using the indirect fluorescent-antibody technique. Magnification, 10,000×.

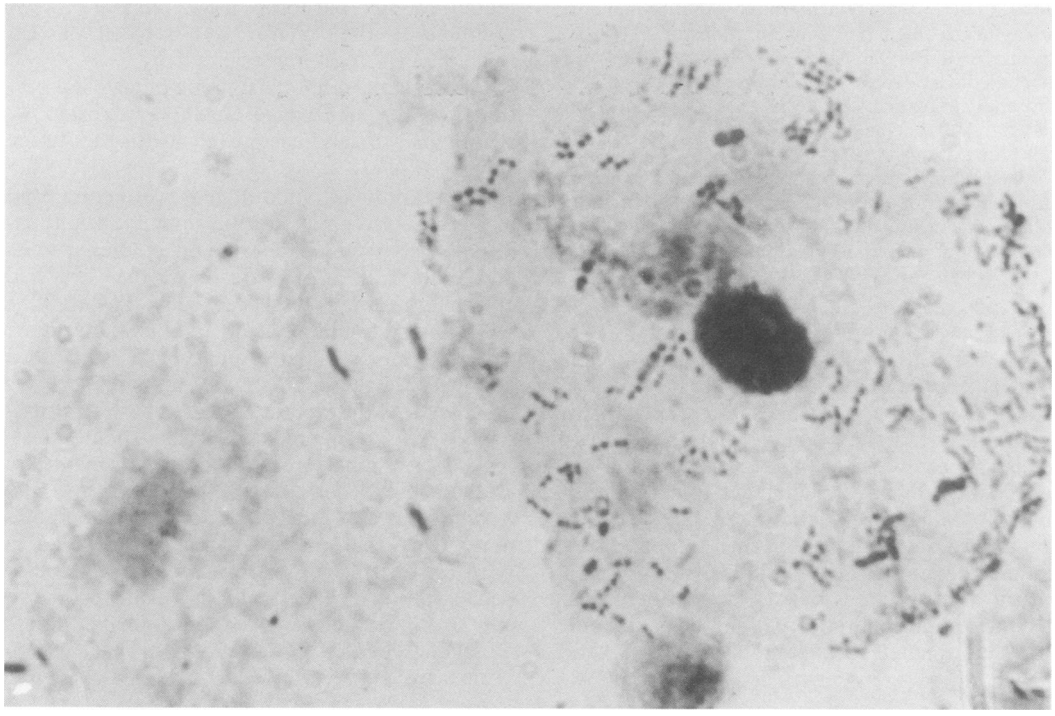

FIG. 2. Photomicrograph of bacteria attached to a mature parakeratotic squamous epithelial cell isolated from the pharynx of a rabbit with an upper respiratory tract infection. The rabbit had been inoculated intranasally with *P. multocida*, and epithelial cells were scraped from the nasal and oral pharynges. Smears of washed cells were stained with May-Grünwald-Giemsa stain. Magnification, 10,000×.

epithelial cells was incubated together at 37°C for 2 h. Incubation at 35°C (temperature of the rabbit nasal passages; H. G. Rush, unpublished observation) had little effect on the numbers of attached bacteria, but no adhesion occurred at 4°C.

Pasteurellae also attached to tissue culture cells (Table 1). HeLa cells proved to be the most, and primary rabbit kidney cells the least, receptive cell line for the attachment of *P. multocida*. HeLa cells, therefore, were used in subsequent assays to characterize the interaction of the bacteria with cell surface membranes.

TABLE 1. Adherence of *P. multocida* strain R-1 to rabbit pharyngeal cells and to tissue culture cells in vitro

Animal cells	Mean adhesion (bacteria per cell ± SD)
Rabbit pharyngeal cells[a]	
Mature squamous cells	44 ± 42
Ciliated cells[b]	0
Tissue culture cells	
HeLa	143 ± 59
Baby hamster kidney	16 ± 11
Vero	75 ± 36
Primary human embryonic lung	22 ± 12
Primary rabbit kidney	12 ± 17

[a] Cells obtained from healthy pasteurella-free animals.

[b] Adhesion to ciliated surface.

Between the limits of 10^7 and 10^9 bacteria per ml, the mean number of bacteria attached per HeLa cell was a linear function of the numbers of bacteria used to challenge a cell monolayer. The higher concentration of bacteria gave a mean of 143 ± 59 bacteria per HeLa cell without saturation of HeLa surfaces, and the lower concentration gave a mean of 2 ± 2 bacteria per HeLa cell. Maximal adhesion occurred at 30 min for all concentrations.

With the exception of the bacteria grown in Bacto-Fildes medium, which were 50% less adhesive, neither the growth medium, phase of growth, nor the condition of cultures had any significant effect on the adhesion of pasteurellae to pharyngeal or tissue culture cells.

Adhesive activity of different *P. multocida* serotypes and isolates. All capsular serotype A strains were adhesive, but adhesiveness was related neither to particular somatic antigens nor to the species from which the pasteurellae had

TABLE 2. Adherence of various *P. multocida* strains to HeLa cells

Designation	Source	Animal origin	Disease[a]	Antigen type		Mean adhesion[b] (bacteria per cell ± SD)
				Somatic	Capsular	
R1	Michigan	Rabbit	RP	3,12	A	100 ± 58
R3	Michigan	Rabbit	RP	12,2	A	29 ± 27
R6	Michigan	Rabbit	RP	12,2,4	A	26 ± 17
R7	Michigan	Rabbit	RP	12,2	A	51 ± 30
R11	Michigan	Rabbit	RP	12,2	A	44 ± 26
R15	Michigan	Rabbit	RP		A	108 ± 75
R25	Michigan	Rabbit	RP	12,2	A	58 ± 31
R27	Michigan	Rabbit	RP	12	A	39 ± 26
R30	Michigan	Rabbit	RP		A	146 ± 108
R71	Michigan	Rabbit	RP		A	83 ± 58
P1062	G. Carter	Bovine	RP	3	A	175 ± 92
P2726	K. Rhoades	Bovine	UNK	12	A	47 ± 41
P2706	K. Rhoades	Bovine	RP	3	A	88 ± 48
P3074	K. Rhoades	Chicken	AS	1	A	104 ± 75
P3170	K. Rhoades	Chicken	UNK	3	A	100 ± 69
P2721	K. Rhoades	Turkey	FC	1	A	30 ± 21
P2791	K. Rhoades	Turkey	FC	10	A	50 ± 36
P2999	K. Rhoades	Cat	NCD	3	A	22 ± 19
P2929	K. Rhoades	Cat	NCD	5	D	37 ± 21
Kobe 6	G. Carter	Swine	RP	12	D	3 ± 2
P3770	R. Rimler/ L. Spanoghe	Rabbit	RP, Absc	3,12,15	D	0.9 ± 1.6
P4066	R. Rimler/ L. Spanoghe	Rabbit	RP	3,12	D	0.5 ± 1.5
P4067	R. Rimler/ L. Spanoghe	Rabbit	S	3,12	D	0.2 ± 0.6
656	G. Carter	Bison	HS	12	B	3 ± 3
Bunia II	G. Carter	Bovine	HS	5,12	E	3 ± 6

[a] RP, Respiratory pasteurellosis; HS, hemorrhagic septicemia; Absc, abscess; S, septicemia; AS, air sacculitis; FC, fowl cholera; NCD, no clinical disease; UNK, unknown.

[b] The number of bacteria per HeLa cell from replicate experiments was normalized to strain R1 which was set at 100.

been isolated originally (Table 2). The range in mean adhesion to HeLa cells of serotype A strains from rabbits was 26 to 100 bacteria per cell, 47 to 175 for bovine strains, 30 to 104 for domestic fowl, and 22 for the single type A cat isolate. With the exception of one serotype D strain (P2929), strains with capsular serotypes B, D, and E were at least 10-fold less adhesive (Table 2). Two serotype D strains (Kobe 6 and P4066), isolated from cases of respiratory disease in other animals, were of the latter nonadhesive type.

Effects of physical and chemical treatments on adhesion. The removal of the hyaluronic acid capsule significantly increased the adhesiveness of three strains of serotype A ($P < 0.0001$), all of which had been isolated from cases of clinical upper respiratory pasteurellosis in rabbits (Table 3). Strain R-1 showed the smallest increase in adhesion and had the smallest capsule of the three strains. The attachment of nonadherent strains was unaffected by hyaluronidase treatment (Table 3).

Heating strain R-1 in a boiling water bath for 10 min reduced adhesion significantly ($P < 0.001$) as did pronase treatment ($P < 0.001$) and homogenization ($P < 0.001$). The latter treatment did not affect viability. Lipases, glycosidases, and the detergent Nonidet P-40 were without significant effect (Table 4).

Characterization of *P. multocida* adhesion by carbohydrate inhibition. Adhesion to HeLa and

TABLE 3. Adherence of decapsulated *P. multocida* to HeLa cells

Strain	Capsule type	Adherence[a]	
		Untreated	Hyaluronidase treated
R1	A	100 ± 64	145 ± 77[b]
R15	A	66 ± 41	136 ± 75[b]
R71	A	59 ± 31	161 ± 73[b]
656	B	5 ± 4	5 ± 5
Kobe 6	D	3 ± 6	3 ± 3
Bunia II	E	4 ± 4	2 ± 2

[a] Bacteria per HeLa cell normalized to untreated strain R1.

[b] Significant at $P < 0.0001$.

TABLE 4. Inhibition of *P. multocida* strain R-1 adhesion to HeLa cells by various treatments

Treatment of bacterium	Mean adhesion[a] (bacteria per cell ± SD)
Untreated control	100 ± 53
Heat (100°C)	74 ± 44[b]
Hydrodynamic shear	44 ± 28[b]
Pronase digestion	58 ± 27[b]
Lipase digestion	82 ± 47
Glycosidase digestion	86 ± 44
Nonidet P-40 extraction	113 ± 53

[a] Bacteria per HeLa cell normalized to untreated control.
[b] Significant at $P < 0.001$.

pharyngeal cells was reduced significantly in the presence of *N*-acetyl-D-glucosamine (Table 5). Adhesion to both cell types was unaffected by D-mannose or by the other four monosaccharides tested (data not shown).

Electron microscopy. Filamentous projections 5 nm in diameter and of variable length were observed on the surfaces of the cells of the adhesive strain R-1 (Fig. 3). Fimbriae were not observed on two nonadherent strains, 656 (serotype B) and Bunia II (serotype E).

DISCUSSION

In experimentally induced respiratory pasteurellosis in rabbits, pasteurellae were associated with the surface cells of the pharyngeal mucosa, but were not observed in the deeper layers of the epithelium. In vivo, bacteria attached to mature parakeratotic squamous epithelial cells of the mucosal surface, but not to ciliated epithelial cells. This selective adhesive ability was reproduced in vitro with broth-grown *P. multocida*, suggesting that the adhesins produced in vivo and in vitro were of the same cell specificities.

The adhesion of *P. multocida* to mature parakeratotic pharyngeal cells and to HeLa cells was similar. The expression of the adhesive property was temperature dependent but not noticeably dependent on growth conditions, although exponential-phase cultures exhibited a slightly higher adhesive ability in all cases (data not shown). More importantly, adhesion to each cell type was inhibited by *N*-acetyl-D-glucosamine, suggesting that similar receptors were utilized on both animal cells. For these reasons and because HeLa cells were the most receptive tissue culture cells examined, HeLa cells were selected as the most convenient and reproducible in vitro model. In this model, serotype A strains were more adhesive than other capsular serotypes, and type A strains isolated from cases of rabbit respiratory pasteurellosis were always adhesive. Such patterns of adhesion might be expected if adhesion is important in the colonization of the rabbit pharyngeal mucosa by serotype A pasteurellae. Type D *P. multocida* strains, which are rare agents of respiratory disease in rabbits (11), were noticeably less adhesive in the HeLa cell model. If adhesion in the pharynx is also characteristic of type D strains, then these results suggest that the adhesive mechanisms of type D and type A strains are different.

P. multocida possesses fimbriae, originally thought to cause twitching of the bacteria (7). The discovery of fimbriae in cultures of an adhesive strain of *P. multocida* but not in cultures of nonadhesive strains suggests that fimbriae may be the adhesive organelle. Furthermore, hydrodynamic shear of *P. multocida*, which may be expected to remove fimbriae, pronase treatment that degrades surface proteins, and heat treatment, which destroys pasteurella hemagglutinating activity (13), all significantly reduced bacterial adhesion. Taken together, these results suggest that the fimbriae mediate adhesion. In contrast, the hyaluronic acid capsule reduces adhesion, possibly because its high-charge density increases repulsion between the animal cell and the bacteria (10), or the capsule may partially mask the adhesin or both. The hyaluronic acid capsule would appear to play no role in attachment to the mucosa, but probably functions in pasteurella infections by increasing resistance to cellular defense mechanisms (12).

The attachment of bacteria to animal cell membranes can involve carbohydrate receptors and adhesion; therefore, bacteria may be inhibited specifically by certain carbohydrates (1). This is the case with *P. multocida*. The inhibitory

TABLE 5. Inhibition of *P. multocida* strain R-1 adhesion to HeLa cells and to pharyngeal epithelial cells by *N*-acetyl-D-glucosamine

Animal cell	Mean adhesion with increasing concn of *N*-acetyl-D-glucosamine[a] (mg/ml)			
	None	0.1	1	5
HeLa	100 ± 40	72 ± 31[b]	54 ± 19[c]	49 ± 24[c]
Squamous pharyngeal	100 ± 57	75 ± 60	97 ± 69	53 ± 45[b]

[a] Bacteria per cell normalized to respective untreated control.
[b] Significant at $P < 0.01$.
[c] Significant at $P < 0.001$.

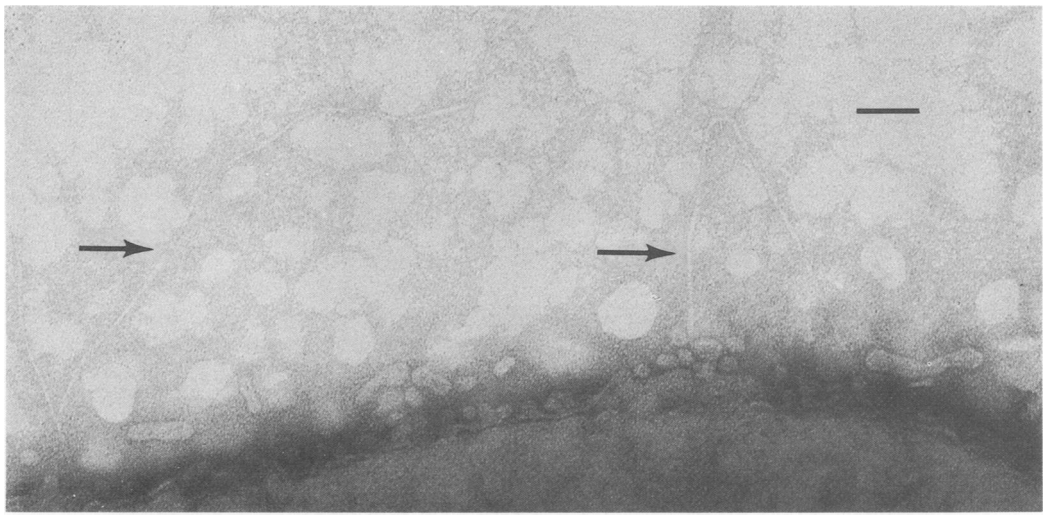

FIG. 3. Electron photomicrograph of *P. multocida* strain R-1 (108,000×). The arrow indicates the fimbria-like projections on the cell surface. The bar represents 50 nm.

action of *N*-acetyl-D-glucosamine suggests that an *N*-acetyl-D-glucosamine residue constitutes at least part of the adhesin receptor on HeLa cells and host pharyngeal cells.

The evidence presented suggests that the attachment of type A *P. multocida* to squamous pharyngeal surfaces results from the interaction of bacterial fimbriae with *N*-acetyl-D-glucosamine receptors of the host cell. This attachment possibly plays a role in colonization and hence pathogenesis of upper respiratory tract infections. Indeed, our recent studies show that inocula containing small numbers of adhesive type A strains of pasteurellae are able to colonize the rabbit pharynx, cause disease after intranasal inoculation, and cause a lethal septicemia by the intravenous route. In contrast, nonadhesive strains of pasteurellae were less pathogenic when introduced into rabbits by either route of inoculation, and the intranasal route produced septicemia rather than causing disease localized to the upper respiratory tract.

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